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***New Phytologist* Supporting Information**

Article title: Rubisco small subunits from the unicellular green alga *Chlamydomonas* complement Rubisco-deficient mutants of Arabidopsis.

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The following Supporting Information is available for this article:

Notes S1 Expression vectors for Rubisco small subunit (*rbcS*) cassettes (see separate Notes S1.zip file). Gateway destination vector pB7WG (Karimi *et al*., 2002) was used for stable Agrobacterium-mediated insertion into Arabidopsis. For fluorescent tag-based localisation in tobacco, *rbcS* genes were fused to a sequence encoding a GFP tag using destination vector pGWB4 (Nakagawa *et al*., 2009), to produce C-terminally GFP-tagged fusion protein.

Fig. S1 Transient expression of Rubisco small subunit-GFP fusion proteins in tobacco.

Fig. S2 Impact of native and heterologous SSUs on photosynthesis and growth in the Arabidopsis mutant *1a3b* background.

Fig. S3 Alignments of the mature Arabidopsis SSU amino acid sequences.

Table S1 Sequences of synthetic oligonucleotides used in this study

Table S2 Transcript abundances of the Rubisco gene family in *rbcs* mutants and transgenic lines

Table S3 Rubisco and soluble protein contents for *rbcs* mutants and transgenic lines

Table S4 Rosette area and biomass for *rbcs* mutants and transgenic lines

Table S5 Chlorophyll characteristics and maximum quantum yield of PSII (F_v/F_m) for *rbcs* mutants and transgenic lines

Table S6 Photosynthetic nonphotochemical quenching capacity for *rbcs* mutants

Fig. S1 Transient expression of Rubisco small subunit-GFP fusion proteins in tobacco. Tobacco (*Nicotiana benthamiana* L.) was cultivated in a glasshouse (minimum 20°C, natural light supplemented to give light periods of at least 12 h). Plants were *c*. 21-d-old at the time of infiltration, and leaves were imaged between 2 and 7 d after infiltration. Native ($1A_{At}$) and heterologous ($1A_{At}$MOD, $S2_{Cr}$) SSUs are shown. Magenta and green signals are chlorophyll autofluorescence and GFP fluorescence respectively. Overlaid images of these signals are shown: overlaps are white. Bar, 25 µm.

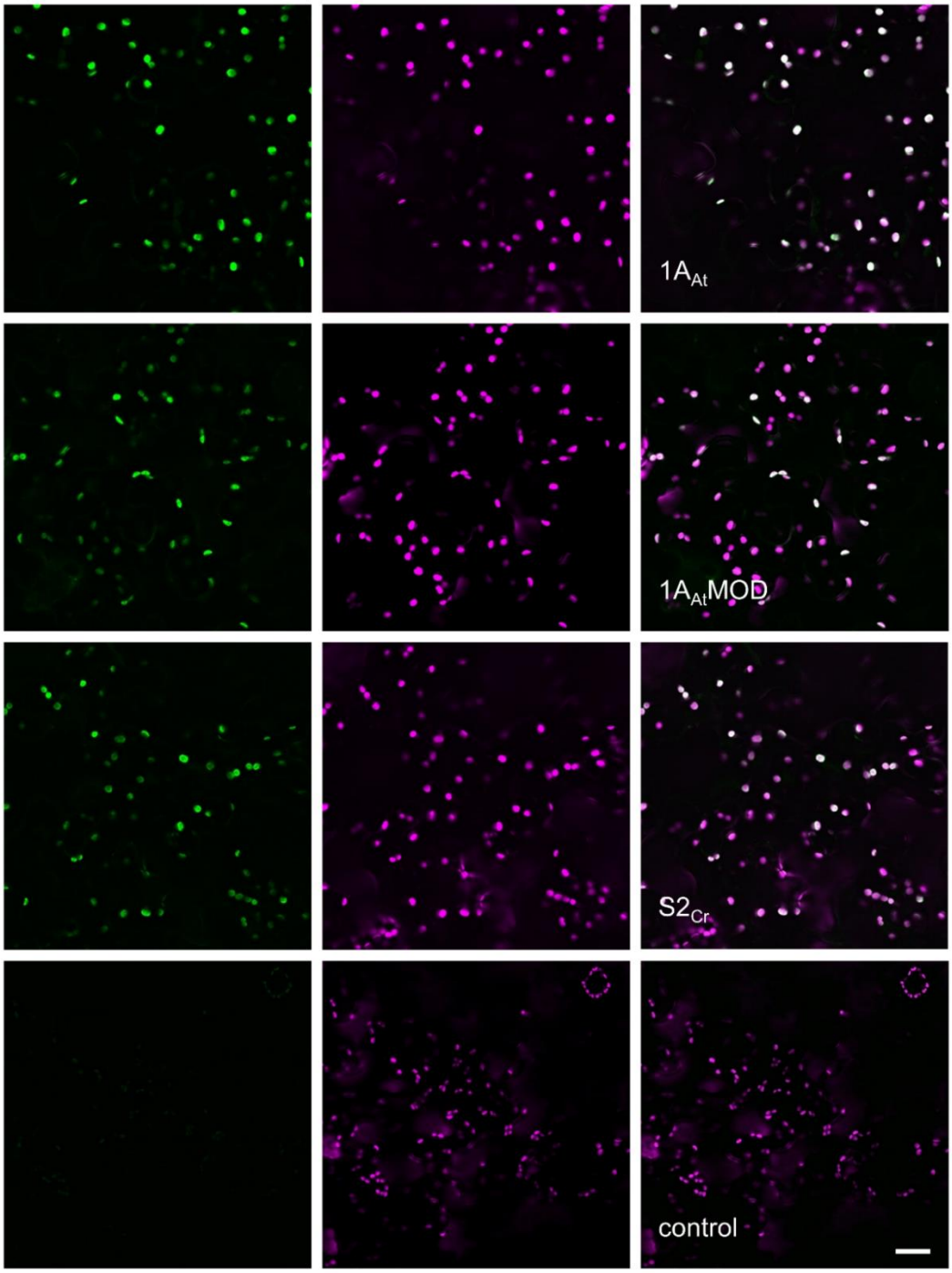

Fig. S2 Impact of native and heterologous SSUs on photosynthesis and growth in the *Arabidopsis thaliana* mutant *1a3b* background. Transgenic lines were screened in the T_2 segregating generation (1:2:1) for differences in growth and maximum quantum yield of PSII (F_v/F_m) relative to *1a3b* mutants and wild-type plants. Values are shown for 45 individual rosettes of wild-type and *1a3b* genotypes, and 15 individual rosettes from each of 6 different transgenic lines (i.e. 90 plants) for $1A_{At}$, $1A_{At}$MOD and $S2_{Cr}$ genotypes.

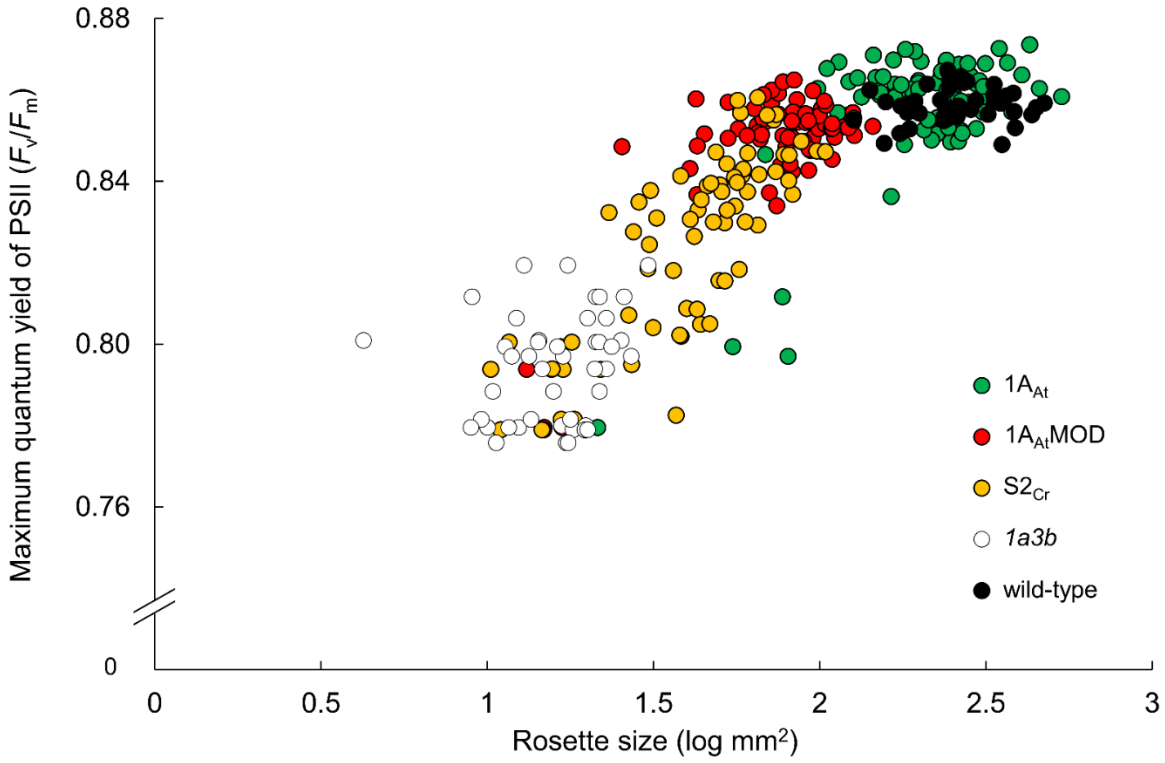

Fig. S3 Alignment of the mature *Arabidopsis thaliana* SSU amino acid sequences. The two α-helixes A and B are highlighted in grey. Differences in amino acid residues between SSUs are in bold and underlined for rbcS1A (1A). Absence of residues is indicated with a dash, and non-conservative differences are marked with a star. Peptides for rbcS1A, rbcS1B (1B), rbcS2B (2B) and rbcS3B (3B) correspond to At1g67090.1, At5g38430.1 At5g38420.1 and At5g38410.1, respectively.

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1A      MQVWPPIGKKKFETLSYLPDLTDSELAKEVDYLIRNKWIPCVEFELEHGFVYREHGNSPG
1B      MKVWPPIGKKKFETLSYLPDLTDVELAKEVDYLLRNKWIPCVEFELEHGFVYREHGNTPG
2B      MKVWPPIGKKKFETLSYLPDLSDVELAKEVDYLLRNKWIPCVEFELEHGFVYREHGNTPG
3B      MKVWPPIGKKKFETLSYLPDLSDVELAKEVDYLLRNKWIPCVEFELEHGFVYREHGNTPG
                              *
1A      YYDGRYWTMWKLPLFGCTDSAQVLKEVEECKKEYPNAFIRIIGFDNTRQVQCISFIAYKP
1B      YYDGRYWTMWKLPLFGCTDSAQVLKEVEECKKEYPGAFIRIIGFDNTRQVQCISFIAYKP
2B      YYDGRYWTMWKLPLFGCTDSAQVLKEVEECKKEYPGAFIRIIGFDNTRQVQCISFIAYKP
3B      YYDGRYWTMWKLPLFGCTDSAQVLKEVEECKKEYPGAFIRIIGFDNTRQVQCISFIAYKP
                                          *
1A      PSFTG-
1B      PSFTDA
2B      PSFTEA
3B      PSFTEA
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Table S1 Sequences of synthetic oligonucleotides used in this study.

Primer ID	Forward primer	Reverse Primer	Amplicon	Reference
rbcS1A (GABI_608F01)	CCATAAGGAAAGGGCCAAGT	CATTGTCCAGTACCGTCCATC	980 bp	signal.salk.edu/tdnaprimers.2.html
rbcS2B (GABI_324A03)	TGGGTTCCTCTTGTTCATCAG	CACTTGTTGCGGAGAAGGTAG	1066 bp	signal.salk.edu/tdnaprimers.2.html
rbcS3B (SALK_117835)	TTTTTAAGAGCATCTCGAATCTATCTC	CACTTGTTGCGGAGAAGGTAG	1160 bp	signal.salk.edu/tdnaprimers.2.html
SALK T-DNA (left border)	ATTTTGCCGATTTCGGAAC	-	-	signal.salk.edu/tdna_FAQs.html
GABI T-DNA (left border)	ATATTGACCATCATACTCATTGC	-	-	www.gabi-kat.de/duplofaq/confirmation-strategy.html
rbcS1A (RT-qPCR)	AATTTCCGGACTTAACGTTTGTTT	CATCAGACAGTTGAGAATCCGATAGA	69 bp	(Izumi *et al.*, 2012)
rbcS1B (RT-qPCR)	GCCAAAGTGAAAAAACTGAAGGTT	AAGAGCAGAAATGAAGTGATATGAATAGA	83 bp	(Izumi *et al.*, 2012)
rbcS2B (RT-qPCR)	ACCCATTTCTATGTGGTCAATGC	TTCACTTTCAAACAATAGTTCCTCAAC	80 bp	(Izumi *et al.*, 2012)
rbcS3B (RT-qPCR)	CCTATTGTCTGTGTTCTTTTTCTCTTTATG	TCAAGACGCACGGATATATAAATTACA	99 bp	(Izumi *et al.*, 2012)
rbcL (RT-qPCR)	GATGGGCTTACCAGCCTTGA	CTGGAACGGGCTCGATGT	61 bp	(Izumi *et al.*, 2012)
$1A_{At}$ and $1A_{At}$MOD (RT-qPCR)	TCATTGCCTACAAGCCACCA	CCGCGGGATATCACCACTTT	85 bp	This work
$S2_{Cr}$ (RT-qPCR)	GTGCAGATCATGGGCTTCCT	TACACGGAGCGCTTGTTGG	77 bp	This work
PP2A (RT-qPCR)	TAACGTGGCCAAAATGATGC	GTTCTCCACAACCGCTTGGT	61 bp	(Czechowski *et al.*, 2005)
At4g26410 (RT-qPCR)	GAGCTGAAGTGGCTTCCATGAC	GGTCCGACATACCCATGATCC	81 bp	(Czechowski *et al.*, 2005)
UBQ10 (RT-qPCR)	AGAACTCTTGCTGACTACAATATCCAG	ATAGTTTTCCCAGTCAACGTCTTAAC	107 bp	This work

Table S2 Transcript abundances of the Rubisco gene family in *rbcs* mutants and transgenic lines of *Arabidopsis thaliana*. Abundances of *rbcS1A*, *rbcS1B*, *rbcS2B*, *rbcS3B* and *rbcL* transcripts were quantified using RT-qPCR with gene-specific primers (Table S1). Values are the means ± SE of measurements made on three 28-d-old rosettes (as shown in Fig. 2). For each subunit, letters above the means ± SE indicate significant difference (P <0.05) as determined by ANOVA followed by Tukey's HSD tests. Values followed by the same letter are not statistically significantly different.

	wild-type	*1a3b*	*1a2b*	$1A_{At}$-1	$1A_{At}$-2	$1A_{At}$-3	$1A_{At}$MOD-1	$1A_{At}$MOD-2	$1A_{At}$MOD-3	$S2_{Cr}$-1	$S2_{Cr}$-2	$S2_{Cr}$-3
rbcS1A	42.8 ± 1.2^{a}	0.08 ± 0.03^{b}	0.05 ± 0.02^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.04 ± 0.03^{b}	0.04 ± 0.02^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}
rbcS1B	8.1 ± 0.2^{a}	5.7 ± 1.6^{a}	9.8 ± 1.7^{a}	6.4 ± 2.5^{a}	10.3 ± 2.3^{a}	9.8 ± 2.2^{a}	8.2 ± 1.4^{a}	6.9 ± 1.8^{a}	4.6 ± 3.5^{a}	4.7 ± 2.8^{a}	7.7 ± 1.6^{a}	9.7 ± 2.4^{a}
rbcS2B	20.9 ± 2.7^{b}	34.0 ± 1.3^{ab}	0.01 ± 0.01^{c}	30.8 ± 8.8^{ab}	22.3 ± 5.2^{ab}	36.3 ± 9.8^{ab}	43.3 ± 8.4^{a}	21.4 ± 5.3^{b}	19.1 ± 3.4^{b}	33.7 ± 3.9^{ab}	42.1 ± 4.3^{a}	34.7 ± 6.3^{ab}
rbcS3B	28.2 ± 3.9^{a}	2.1 ± 0.8^{b}	31.7 ± 10.6^{a}	0.3 ± 0.1^{b}	0.1 ± 0.1^{b}	3.1 ± 0.9^{b}	0.5 ± 0.4^{b}	0.4 ± 0.2^{b}	0.5 ± 0.2^{b}	0.5 ± 0.4^{b}	0.2 ± 0.1^{b}	0.1 ± 0.1^{b}
rbcL	100 ± 6.1^{a}	48 ± 6.1^{c}	45 ± 2.1^{c}	86.2 ± 7.7^{ab}	94.5 ± 4.7^{ab}	109 ± 12^{a}	95.0 ± 3.6^{ab}	82.8 ± 9.2^{b}	92.1 ± 4.4^{ab}	98.3 ± 2.1^{a}	94.3 ± 5.2^{ab}	91.5 ± 2.1^{ab}
$1A_{At}$		-	-	45.1 ± 1.8^{a}	45.7 ± 4.2^{a}	43.3 ± 1.4^{a}	-	-	-	-	-	-
$1A_{At}$MOD		-	-	-	-	-	42.3 ± 2.1^{a}	46.7 ± 0.7^{a}	47.1 ± 3.3^{a}	-	-	-
$S2_{Cr}$		-	-	-	-	-	-	-	-	39.2 ± 1.7^{a}	42.2 ± 1.5^{a}	42.5 ± 0.8^{a}

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Table S3 Rubisco and soluble protein contents for *rbcs* mutants and transgenic lines of *Arabidopsis thaliana*. Rubisco content was determined via ^{14}C-CABP binding, subunit ratios were estimated by immunoblotting. Values are the means ± SE of measurements made on leaf samples from three 32-d-old rosettes (as shown in Fig. 3) followed by letters indicating significant difference (P <0.05) as determined by ANOVA followed by Tukey's HSD tests. Values followed by the same letter are not statistically significantly different.

	WT	*1a3b*	*1a2b*	$1A_{At}$-1	$1A_{At}$-2	$1A_{At}$-3	$1A_{At}$MOD-1	$1A_{At}$MOD-2	$1A_{At}$MOD-3	$S2_{Cr}$-1	$S2_{Cr}$-2	$S2_{Cr}$-3
Total Rubisco (g m^{-2})	0.86 ± 0.05^{a}	0.26 ± 0.08^{e}	0.42 ± 0.06^{de}	0.86 ± 0.7^{a}	0.58 ± 0.06^{bcd}	0.48 ± 0.1^{bcd}	0.53 ± 0.04^{bcd}	0.61 ± 0.1^{abcd}	0.76 ± 0.08^{ab}	0.56 ± 0.07^{bcd}	0.47 ± 0.07^{cd}	0.66 ± 0.06^{abc}
Soluble protein (g m^{-2})	2.9 ± 0.2^{a}	1.6 ± 0.2^{d}	1.9 ± 0.1^{cd}	2.6 ± 0.1^{ab}	2.5 ± 0.2^{ab}	2.4 ± 0.1^{abc}	2.4 ± 0.1^{abc}	2.6 ± 0.2^{ab}	2.8 ± 0.1^{ab}	2.5 ± 0.1^{ab}	2.3 ± 0.1^{abc}	2.6 ± 0.1^{ab}
LSU (g m^{-2})	0.68 ± 0.3^{a}	0.21 ± 0.05^{e}	0.34 ± 0.04^{de}	0.67 ± 0.05^{a}	0.45 ± 0.04^{bcd}	0.38 ± 0.07^{bcd}	0.42 ± 0.03^{bcd}	0.48 ± 0.08^{abcd}	0.59 ± 0.06^{ab}	0.44 ± 0.06^{bcd}	0.37 ± 0.05^{cd}	0.52 ± 0.05^{abc}
Total SSU (g m^{-2})	0.2 ± 0.01^{a}	0.05 ± 0.01^{e}	0.09 ± 0.01^{de}	0.18 ± 0.02^{ab}	0.13 ± 0.01^{bcd}	0.11 ± 0.03^{bcd}	0.12 ± 0.01^{bcd}	0.15 ± 0.03^{abc}	0.18 ± 0.02^{ab}	0.12 ± 0.02^{cd}	0.1 ± 0.01^{cd}	0.15 ± 0.01^{abc}
LSU: SSU (g: g)	3.4 ± 0.2^{a}	3.9 ± 0.2^{a}	3.8 ± 0.2^{a}	3.8 ± 0.3^{a}	3.4 ± 0.2^{a}	3.3 ± 0.2^{a}	3.6 ± 0.2^{a}	3.2 ± 0.3^{a}	3.2 ± 0.2^{a}	3.9 ± 0.3^{a}	3.7 ± 0.2^{a}	3.5 ± 0.3^{a}
Native SSU (g m^{-2})	0.2 ± 0.01^{a}	0.05 ± 0.01^{e}	0.09 ± 0.01^{cd}	0.18 ± 0.02^{ab}	0.13 ± 0.01^{bc}	0.11 ± 0.03^{cd}	0.04 ± 0.01^{e}	0.05 ± 0.01^{e}	0.04 ± 0.01^{e}	0.07 ± 0.01^{de}	0.06 ± 0.01^{e}	0.08 ± 0.01^{de}
Heterologous SSU (g m^{-2})	-	-	-	-	-	-	0.07 ± 0.01^{bc}	0.10 ± 0.03^{ab}	0.14 ± 0.02^{a}	0.05 ± 0.01^{c}	0.04 ± 0.01^{c}	0.06 ± 0.01^{bc}

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Table S4 Rosette area and biomass for *rbcs* mutants and transgenic lines of *Arabidopsis thaliana*. Values are the means ± SE of measurements made on ten 28-d-old rosettes (as shown in Fig. 4). Letters above the means ± SE indicate significant difference (P <0.05) as determined by ANOVA followed by Tukey's HSD tests. Values followed by the same letter are not statistically significantly different. Abbreviations: FW, fresh weight; DW, dry weight; SLA, specific leaf area.

	WT	*1a3b*	*1a2b*	$1A_{At}$-1	$1A_{At}$-2	$1A_{At}$-3	$1A_{At}$MOD-1	$1A_{At}$MOD-2	$1A_{At}$MOD-3	$S2_{Cr}$-1	$S2_{Cr}$-2	$S2_{Cr}$-3	$1A_{At}$-1 seg	$1A_{At}$MOD-1 seg	$S2_{Cr}$-1 seg
FW (g)	0.27 ± 0.01^{a}	0.025 ± 0.003^{fg}	0.06 ± 0.01^{ef}	0.24 ±0.01^{ab}	0.24 ± 0.01^{ab}	0.2 ± 0.01^{bcd}	0.19 ± 0.01^{cd}	0.19 ± 0.01^{cd}	0.22 ± 0.01^{bc}	0.1 ± 0.01^{e}	0.16 ± 0.01^{d}	0.17 ± 0.01^{d}	0.02 ± 0.01^{g}	0.01 ± 0.01^{g}	0.01 ± 0.01^{g}
DW (mg)	24.5 ± 1.0^{a}	2.0 ± 0.1^{g}	6.1 ± 0.4^{f}	22.0 ±1.2^{ab}	21.4 ± 1.2^{abc}	18.5 ± 1.1^{bcde}	17.6 ± 1.0^{cde}	17.0 ± 1.0^{de}	20.3 ± 0.7^{bcd}	9.3 ± 0.4^{ef}	15.5 ± 0.9^{e}	16.3 ± 0.8^{e}	1.1 ± 0.1^{g}	1.0 ± 0.1^{g}	0.7 ± 0.1^{g}
FW/DW	11.2 ± 0.4^{ab}	12.8 ± 0.8^{a}	9.6 ± 0.1^{b}	10.8 ±0.3^{ab}	11.0 ± 0.3^{ab}	10.6 ± 0.1^{ab}	10.7 ± 0.1^{ab}	10.9 ± 0.1^{ab}	10.7 ± 0.1^{ab}	11.1 ± 0.6^{ab}	10.3 ± 0.1^{ab}	10.5 ± 0.1^{ab}	11.9 ± 1.3^{ab}	13.0 ± 1.0^{a}	12.1 ± 1.0^{ab}
Area (mm^2)	491 ± 14^{a}	89 ± 6^{d}	284 ± 21^{c}	487 ±23^{a}	455 ± 23^{ab}	433 ± 28^{ab}	445 ± 21^{ab}	411 ± 24^{ab}	458 ± 13^{ab}	273 ± 15^{c}	383 ± 19^{b}	437 ± 25^{ab}	50 ± 6^{d}	60 ± 3^{d}	47 ± 4^{d}
Area/FW (m^2 kg^{-1})	1.8 ± 0.1^{c}	3.7 ± 0.22^{b}	4.9 ± 0.4^{a}	2.1 ±0.1^{c}	2.0 ± 0.1^{c}	2.2 ± 0.1^{c}	2.3 ± 0.1^{c}	2.2 ± 0.1^{c}	2.1 ± 0.1^{c}	2.7 ± 0.2^{bc}	2.4 ± 0.1^{c}	2.6 ± 0.1^{bc}	5.1 ± 0.3^{a}	5.0 ± 0.3^{a}	4.8 ± 0.3^{a}
SLA (m^2 kg^{-1}) Area/DW	20 ± 0.1^{c}	47 ± 0.2^{b}	47 ± 0.4^{b}	22 ±0.1^{c}	21 ± 0.1^{c}	23 ± 0.1^{c}	25 ± 0.1^{c}	24 ± 0.1^{c}	23 ± 0.1^{c}	29 ± 0.1^{c}	25 ± 0.1^{c}	27 ± 0.1^{c}	48 ± 0.5^{ab}	63 ± 0.3^{a}	58 ± 0.4^{ab}

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Table S5 Chlorophyll contents and maximum quantum yield of PSII (F_v/F_m) for *rbcs* mutants and transgenic lines of *Arabidopsis thaliana*. Values are the means ± SE of measurements made on four 28-d-old rosettes for chlorophyll and ten 28-d old rosettes for F_v/F_m rosettes. F_v/F_m is shown for attached leaves dark-adapted for 45 min prior to fluorescence measurements. Letters above the means ± SE indicate significant difference (P <0.05) as determined by ANOVA followed by Tukey's HSD tests. Values followed by the same letter are not statistically significantly different.

	WT	*1a3b*	*1a2b*	$1A_{At}$-1	$1A_{At}$-2	$1A_{At}$-3	$1A_{At}$MOD-1	$1A_{At}$MOD-2	$1A_{At}$MOD-3	$S2_{Cr}$-1	$S2_{Cr}$-2	$S2_{Cr}$-3
Chl a (µmol m^{-2})	185 ± 19^a	82±4^b	205 ± 3^a	179 ± 3^a	158 ± 12^a	197 ± 13^a	209 ± 12^a	176 ± 13^a	181 ± 5^a	174 ± 10^a	202 ± 13^a	173 ± 9^a
Chl b (µmol m^{-2})	59 ± 5^a	26±2^b	62 ± 2^a	59 ± 1^a	52 ± 4^a	63 ± 2^a	62 ± 3^a	60 ± 2^a	61 ± 2^a	52 ± 3^a	63 ± 2^a	57 ± 1^a
Chl a+b (µmol m^{-2})	245 ± 24^a	108±5^b	267 ± 3^a	239 ± 3^a	211 ± 16^a	260 ± 15^a	271 ± 15^a	235 ± 15^a	242 ± 4^a	225 ± 13^a	265 ± 14^a	231 ± 9^a
Chl a/b ratio	3.1 ± 0.1^a	3.2±0.1^a	3.3 ± 0.1^a	3.0 ± 0.1^a	3.0 ± 0.1^a	3.1 ± 0.1^a	3.4 ± 0.1^a	2.9 ± 0.1^a	3.0 ± 0.2^a	3.4 ± 0.1^a	3.2 ± 0.1^a	3.0 ± 0.1^a
F_v/F_m	0.854 ± 0.001^a	0.764 ± 0.008^b	0.856 ± 0.01^a	0.850 ± 0.001^a	0.85 ± 0.002^a	0.85 ± 0.001^a	0.846 ± 0.002^a	0.841 ± 0.002^a	0.852 ± 0.001^a	0.846 ± 0.001^a	0.848 ± 0.001^a	0.849 ± 0.001^a

Table S6 Photosynthetic nonphotochemical quenching capacity for *rbcs* mutants of *Arabidopsis thaliana*. Total NPQ was measured after 45 min exposure to high light (600 µmol photons m^{-2} s^{-1}) and after 1 h darkness. Rapidly relaxing NPQ (NPQ_{slow}) and slowly relaxing NPQ (NPQ_{fast}) were quantified according to Griffiths & Maxwell (1999). Values are the means ± SE of measurements on individual leaves from three different rosettes, followed by letters indicating significant difference (P <0.05), as determined by ANOVA followed by Tukey's HSD tests. Values followed by the same letter are not statistically significantly different.

	WT	*1a3b*	*1a2b*
NPQ capacity	3.39 ± 0.32^{b}	4.33 ± 0.28^{a}	2.19 ± 0.21^{c}
% NPQ_{fast} (qE)	1.84 ± 0.11^{b} (55%)	3.39 ± 0.4^{a} (78%)	0.72 ± 0.25^{c} (32%)
% NPQ_{slow} (qI)	1.55 ± 0.32^{a} (45%)	0.94 ± 0.28^{c} (22%)	1.47 ± 0.1^{b} (68%)

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